Monatshefte für Chemie 127, 811–820 (1996)

Monatshefte für Chemie
Chemical Monthly

Mixed-Ligand Complexes of Palladium(II) Involving Propane 1,2,3-triamine as Primary Ligand and Amino Acids, Peptides, or DNA Units as Secondary Ligands

M. M. Shoukry[1], **S. M. El-Medani**[2], and **M. M. Khatab**[2]

[1] Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt
[2] Department of Chemistry, Faculty of Science, Cairo University, Fayoum, Egypt

Summary. The acid-base equilibria of propane 1,2,3-triamine (*PTA*) and the formation equilibria of binary and ternary complexes of palladium(II) with *PTA* as primary ligand and amino acid, peptide, and DNA subunits as secondary ligand have been investigated. Pd(II) is found to form a 1:1 complex with *PTA*. The ternary complexes are formed in a stepwise mechanism, whereby *PTA* first binds to Pd(II) and then ligates to the secondary ligand. The hydrolysis of the Pd-*PTA* complex and the deprotonation of the amide residues in the peptide complexes are discussed in relation to physiological conditions.

Keywords. Mixed-ligand complexes of palladium(II); Pd(II) complexes with propane 1,2,3-triamine; Amino acids; Peptides; DNA; Stability constants; Potentiometric studies.

Gemischte Palladium(II)-Komplexe mit Propan-1,2,3-triamin als Primärligand und Aminosäuren, Peptiden oder DNA-Einheiten als Sekundärliganden

Zusammenfassung. Das Säure-Basen-Gleichgewicht von Propan-1,2,3-triamin (*PTA*) und die Bildungsgleichgewichte von binären und ternären Komplexen von Pd(II) mit *PTA* als Primärligand und Aminosäuren, Peptiden und DNA-Einheiten als Sekundärliganden wurden untersucht. Pd(II) bildet mit *PTA* einen 1:1-Komplex. Die ternären Komplexe werden stufenweise gebildet, wobei *PTA* zuerst an Pd(II) koordiniert; dann erfolgt die Bindung an den Sekundärliganden. Die Hydrolyse des Pd-*PTA*-Komplexes und die Deprotonierung der Amide in den Peptidkomplexen werden im Zusammenhang mit physiologischen Bedingungen diskutiert.

Introduction

Platinum group metal complexes of nucleic acid bases and their derivative have attracted considerable attention because of their antitumour and antibacterial activity [1–5]. The discovery of *cis*-platinum as a cancer chemotherapeutic agent [6] aroused much interest in equilibrium studies. The biological activity of *cis*-platinum is due to its ability to bind the guaninecytosine region of the DNA strand and stop the replication process [7, 8].

Platinum(II) and palladium(II) form square planar complexes forming *cis* and *trans* isomers. The *cis*-complexes are very active. Amino acids are biologically important molecules and do not possess toxicity. Several ternary complexes of platinum(II) and palladium(II) with amino acids and purines, pyrimidines, and nucleosides have been reported [9–13]; some of them are biologically active against human pathogens [14].

In view of the importance of this class of complexes in the chemotherapy of tumours and in continuation of our research program directed to study palladium(II) complexes of expected antitumour activity [15–19], the present investigation traces the formation and characteristics of palladium(II) complexes involving propane 1,2,3-triamine and some selected amino acids, peptides, and DNA units. The most plausible structure of the formed chelate can be assigned on the basis of molecular models. Examining the *Newman* projection formula of propane 1,2,3-triamine indicates that *PTA* will behave as a bidentate like ethylenediamine. The unbound methylamino group may favour hydrogen bonding to DNA units and this may stabilize the metal-DNA complexes.

Results and Discussion

Protonation equilibria

The potentiometric titration curve of the protonated ligand H_3L^{3+} shows three deprotonation steps in the *pH* range of 3.4–10.5. The titration data indicate the presence of simple H_3L^{3+}, H_2L^{2+}, and HL^{+} complexes. The potentiometric titration curves (calculated (line) and observed (symbol)) of the completely protonated form of the ligand are shown in Fig. 1. The concentration distribution diagram of the various protonated forms of the ligand is shown in Fig. 2.

Palladium(II)-PTA equilibria

The titration data obtained were evaluated taking into consideration all feasible models. The equilibrium patterns were selected by successive attempts according to the best agreement between observed and calculated data and by means of an accurate statistical analysis of the agreement factor (σ^2), the goodness of fit (x^2), the standard deviation (σ) of the formation constant, and the chemical significance of the species proposed. At this point, all protonation constants were kept constant, and the computer program MINIQUAD was employed for a second stage of refinement. The accepted model consists of species Pd*L*. After complete formation of the Pd*L* complex, the titration curve drifts due to the formation of the hydroxo complexes. The data in this region are fitted considering the formation of $Pd(PTA)(OH)_n$ species where $n = 1$ and 2. The concentration distribution diagrams of the Pd(*PTA*)–(OH) system is given in Fig. 3. The concentration of the *mono*-hydroxo species increases with increasing *pH*, attaining a maximum of 100% at a *pH* range of 7–9. Further increase in *pH* is accompanied by a decrease in the *mono*-hydroxo species concentration and an increase in the *di*-hydroxo species concentration. This reveals that in the physiological *pH* range the *mono*-hydroxo complex is the predominant species and can interact with bioligands as DNA subunits.

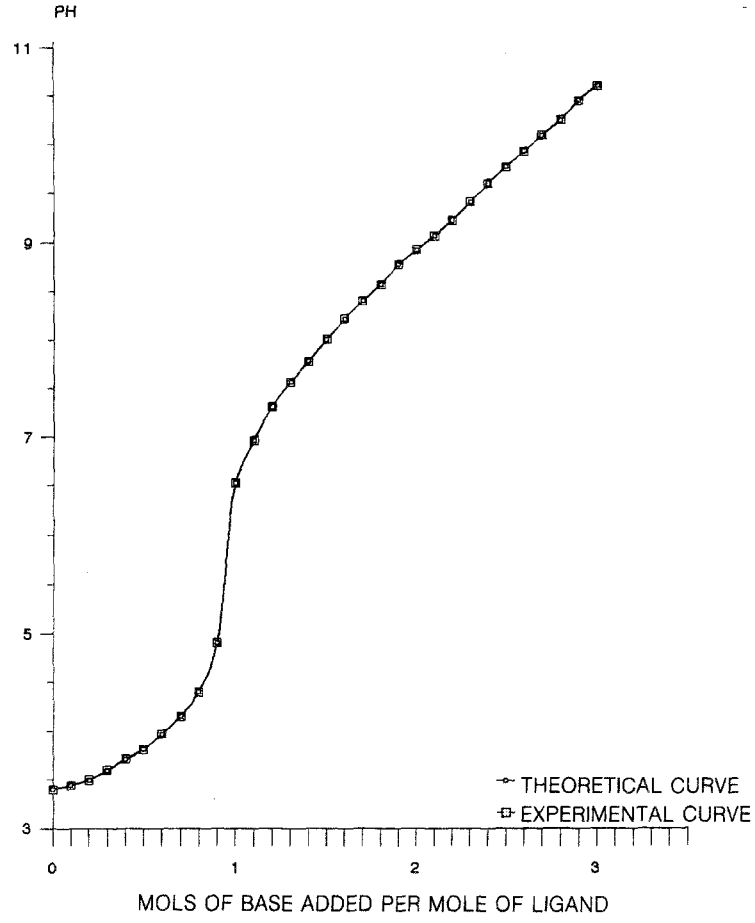

Fig. 1. Potentometric titration curves for the *PTA* system

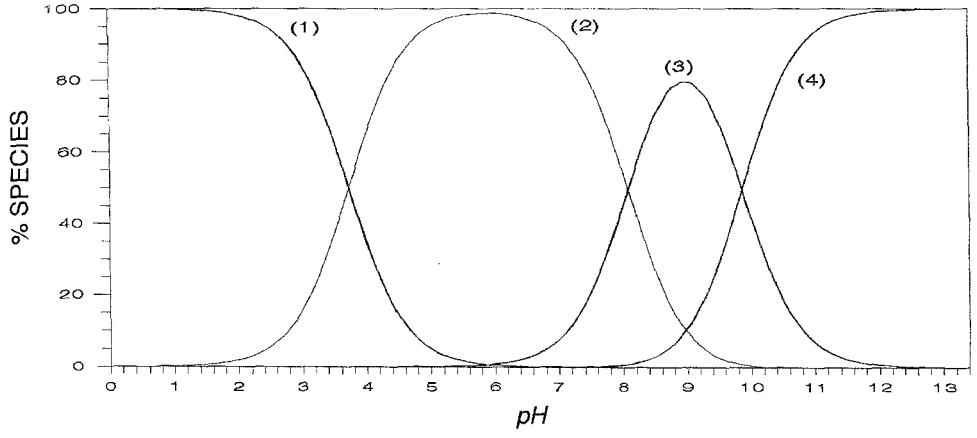

Fig. 2. Concentration distribution diagram of the various protonated forms of propane 1,2,3-triamine; curves: (1) H_3L^{3+}; (2) H_2L^{2+}; (3) HL^{+}; (4) L

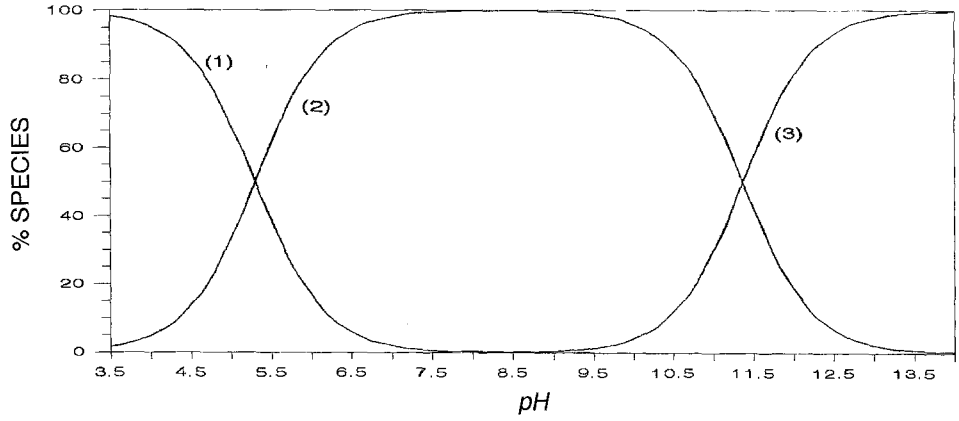

Fig. 3. Concentration distribution diagram of the Pd(*PTA*)-(OH) system; curves: (1) Pd(*PTA*); (2) Pd(*PTA*)(OH); (3) Pd(*PTA*)(OH); (3) PD(*PTA*)$(OH)_2$

Ternary Pd(II) complexes

The potentiometric titration curves of the Pd-*PTA* complex in the presence and absence of glycine are given in Fig. 4 as an example. The formation of a ternary complex is ascertained by comparison of the mixed ligand titration curve with the composite curve obtained by graphical addition of the glycine titration data to the Pd-*PTA* titration curve. The mixed ligand system was found to deviate from the resultant composite curve, indicating the formation of a ternary complex. Based on the above findings and since 1:1 Pd(II)-*PTA* complexes are appreciably more stable the 1:1 Pd(II)-secondary ligand complexes (amino acids, peptides, or DNA) [20], it seems evident that in presence of both ligands *PTA* is primarily ligated to the Pd(II) ion, occupying two coordination positions. This is followed by ligation of the secondary ligand, occupying the remaining coordination positions.

Histidine is a tridentate ligand and may coordinate in a glycine-like or histamine-like way. The stability constant value of the histidine complex is higher than that of α-amino acids and close to that of histamine. This indicates that histidine is coordinating in the histamine-like way. Histidine, histamine, cysteine, and penicillamine (*A*) are protonated in addition to the deprotonated complex species. The acid dissociation constant of the protonated species is given in Ref. [21].

$$pK^{(H)}_{Pd(PTA)(A)(H)} = \log K^{Pd(PTA)}_{Pd(PTA)(A)(H)} - \log K^{Pd(PTA)}_{Pd(PTA)(A)}$$

The pK^H value for the histidine complex is 7.11. This value is between that of protonated imidazole ($pK^H = 6.1$) and the ammonium group ($pK^H = 9.05$). This indicates that the proton in the protonated complex is located partly on the amino group and partly on the imidazole group, *i.e.* two isomers of the protonated complex exist in equilibrium. The acid dissociation constants of the protonated complexes of cysteine and penicillamine are 8.68 and 8.54, respectively. These values compare fairly with their first macroscopic acid dissociation constant (pK^H) amounting to 7.86 and 8.16 (Table 1). This indicates that the proton of the protonated complex will be located partly at the NH_2 group and partly at the S^- group, *i.e.* two isomers of the

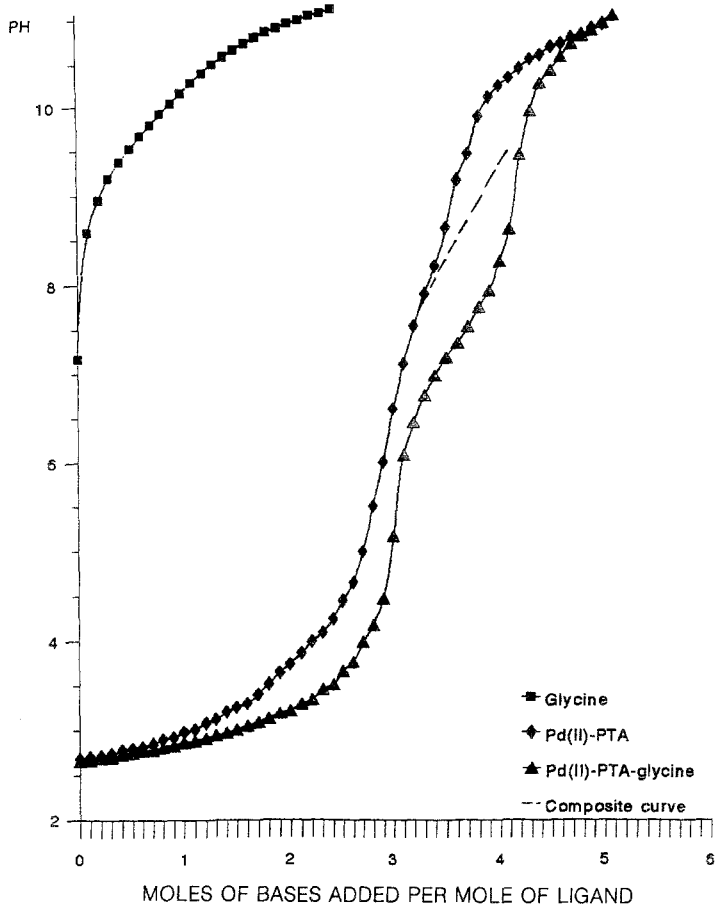

Fig. 4. Potentometric titration curves of Pd(II)-*PTA*-glycine system

protonated complex exist in equilibrium [22]. Further investigation of the complex formation equilibria and these ligands, containing SH and NH_2 groups, will be performed by NMR measurements to shed more light on the complex formation ability of these groups.

The peptides glycyl-valine and leucyl-alanine may coordinate through the terminal amino and carboxyl groups with the amide group in the side chain. The ligands would then behave like a simple amino acid. Alternatively, they can also coordinate through the terminal amino group and the carbonyl oxygen of the amide group. A third possibility, involving the carboxyl and the amide group, can be safely ruled out in view of the great affinity of palladium to nitrogen donor centers. There is no conclusive evidence so far to exclude one or the other of these possibilities. From the fact that $\log \beta_{110}$ for the peptide complexes compare favourably with that of α-amino acids, if the difference in their basicities are considered, one can assume that the peptides probably coordinate as simple amino acids. The pK_a of the peptide complexes ($\log \beta_{110} - \log \beta_{11-1}$) are 8.21 and 8.02 for glycyl-valine and leucyl-alanine, respectively. These values are higher than those of $Pd(en)^{2+}$ complexes with peptides [23]. The stability of the peptide complexes provides the driving force for the deprotonation. The higher pK_a values of the peptide complexes under investiga-

Table 1. Formation constants of mixed ligand complexes

System	*l*	*p*	*q*[a]	log *B*[b]	*S*[c]
PTA-H^+	0	1	1	9.86(0.01)	1.2×10^{-7}
	0	1	2	17.93(0.02)	
	0	1	3	21.65(0.03)	
(Pd-*PTA*)-OH	1	0	−1	−5.30(0.04)	4.9×10^{-8}
	1	0	−2	−16.67(0.05)	
Glycine	0	1	1	9.45(0.00)	1.0×10^{-9}
	1	1	0	6.16(0.04)	2.0×10^{-7}
Alanine	0	1	1	9.59(0.00)	7.7×10^{-7}
	1	1	0	6.38(0.04)	2.4×10^{-7}
Proline	0	1	1	10.49(0.01)	3.8×10^{-8}
	1	1	0	6.58(0.02)	1.1×10^{-7}
Methionine	0	1	1	9.12(0.00)	1.6×10^{-8}
	1	1	0	6.09(0.03)	1.8×10^{-7}
Imidazole	0	1	1	7.07(0.00)	3.1×10^{-8}
	1	1	0	4.22(0.01)	8.6×10^{-9}
Inosine	0	1	1	8.78(0.00)	2.6×10^{-8}
	1	1	0	3.96(0.06)	2.2×10^{-8}
Guanosine	0	1	1	8.88(0.00)	2.3×10^{-8}
	1	1	0	4.43(0.06)	2.2×10^{-8}
Cysteine	0	1	1	10.15(0.02)	7.8×10^{-8}
	0	1	2	18.31(0.03)	
	1	1	0	5.79(0.04)	5.4×10^{-8}
	1	1	1	14.45(0.05)	
Pencillamine	0	1	1	10.41(0.02)	2.6×10^{-7}
	0	1	2	18.27(0.04)	
	1	1	0	6.05(0.04)	5.1×10^{-8}
	1	1	1	14.64(0.04)	
Histidine	0	1	1	9.05(0.01)	1.6×10^{-7}
	0	1	1	15.15(0.01)	
	1	1	0	8.52(0.05)	5.2×10^{-8}
	1	1	1	15.63(0.04)	
Histamine	0	1	1	9.84(0.01)	1.1×10^{-7}
	0	1	2	16.07(0.01)	
	1	1	0	8.85(0.06)	1.1×10^{-7}
	1	1	1	15.34(0.05)	
Glycyl-L-valine	0	1	1	8.24(0.01)	2.8×10^{-8}
	1	1	0	4.65(0.07)	2.6×10^{-7}
	1	1	−1	−4.43(0.08)	
DL-leucyl-*DL*-alanine	0	1	1	7.70(0.01)	8.8×10^{-8}
	1	1	0	4.72(0.07)	2.7×10^{-7}
	1	1	−1	−3.70(0.07)	

[a] Symbols l, p, q are the stoichiometric coefficients corresponding to Pd(II)-*PTA*, ligand, and H^+, respectively; [b] standard deviations are given in parentheses; [c] sum of square of residuals

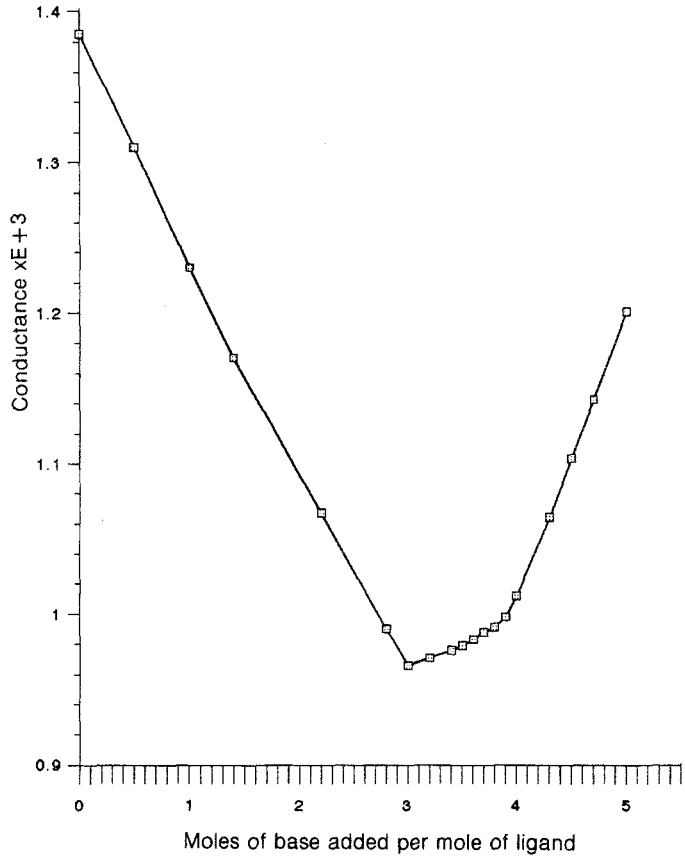

Fig. 5. Conductometric titration curve of Pd-(II)-*PTA*-Glycine

tion are due to their relatively lower stability compared to $Pd(en)^{2+}$ analogs. The lower stability is a result of the steric interaction between the aminomethyl group of *PTA* and the bulky alkyl group of the entering peptide.

The relative magnitudes of pK_a of the peptide complexes have interesting biological applications. Under normal physiological conditions (*pH ca.* 7.4), $[Pd(PTA)]^{2+}$ can catalyze the deprotonation of the amide group. The catalytic action would be very specific under physiological condition. The slight difference in the side chain of the peptides will produce dramatic differences in their behaviour towards the palladium species.

Mixed ligand complexes of Pd-*PTA* with the nucleosides inosine and guanosine were investigated. Their formation constants, given in Table 1, are smaller than those of amino acids. This reveals that the amino acids will compete with DNA for the reaction with $Pd(PTA)^{2+}$ complexes.

The conductometric titration curve for the ternary complex of Pd(II) with *PTA* and glycine (Fig. 5) shows an initial decrease and an inflection at $a = 3$ which probably corresponds to the neutralization of H^+ ions resulting from the formation of Pd(II)-*PTA*. In the $4 \geqslant a \geqslant 3$ range, the conductance increases slightly, supposedly due to the formation of a ternary complex associated with the release of a H^+ ion from glycine. Beyond $a = 4$, the conductance increases appreciably due to the presence of an excess of NaOH.

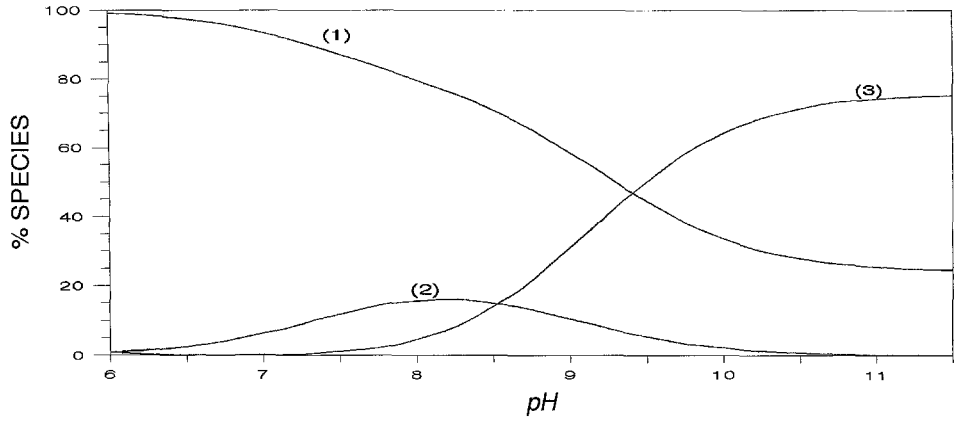

Fig. 6. Concentration distribution diagram of the Pd(II)-*PTA*-Penicillamine system; curves: (1) Pd(*PTA*); (2) Pd(*PTA*)(H*A*); and (3) Pd(*PTA*)(*A*)

The equilibrium concentration distribution diagrams of the various complex species provide a useful picture of palladium(II) binding in the biological system. In all species investigated the concentration of the complex increases with increasing *pH*. That makes complex formation more favourable in the physiological *pH* range. The protonated ternary complex species have been found to be most favoured at lower *pH* values. In order to indicate the main features observed in the species distribution plots in these system, the speciation diagram obtained for Pd-*PTA*-penicillamine is shown in Fig. 6.

Conclusions

In combination of the data of the ligands of various functional groups, it seems to be possible to calculate the equilibrium distribution of the metal species in solutions where the ligands of various classes are present simultaneously. This would form a clear basis for understanding the mode of action of such metal species under physiological conditions.

Experimental

Materials and Reagents

Propane 1,2,3-triamine·3HCl (*PTA*·3HCl) was prepared as described previously [24]. According to its elemental analysis, it was sufficiently pure. Glycine, alanine, proline, methionine, histidine·HCl, histamine·2HCl, penicillamine, cysteine, imidazole, inosine, guanosine, glycyl-*L*-valine, and *DL*-leucyl-*DL*-alanine were obtained from Sigma. K_2PdCl_4 was received from Aldrich. The concentration of a stock solution of *PTA*·3HCl was checked potentiometrically. A solution of K_2PdCl_4 was prepared and estimated complexometrically [25]. The NaOH solution used for the titrations was determined with potassium hydrogen phthalate (Merck Chem. Co.). All solutions were prepared in deionized water.

Apparatus

The potentiometric *pH* titrations were carried out on a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland). The electrode was calibrated with standard buffer solutions prepared according to NBS specifications [26]. The electrolytic conductance was measured using a WTW LBP conductivity bridge (Germany).

Procedures and measuring techniques

The acid dissociation constants of the ligands in the protonated forms were determined by titrating 40 ml of aqueous ligand solution (1.25×10^{-3} *M*) and $NaNO_3$ (0.1 *M*). The conditions of measurements for the determination of formation constant of the Pd-*PTA* complex were the same as before, but part of $NaNO_3$ was replaced by K_2PdCl_4 in the ratio Pd^{2+}:*PTA* = 1:1. The conditions of measurements for the titration of the ternary complexes were the same as for the binary one, but the solutions contained equivalent amounts of *PTA*, Pd(II), and the other ligand (*A*). The stability constants $K^{Pd(PTA)}_{Pd(PTA)(A)}$ for the ternary complex were determined using the data obtained within the *pH* range corresponding to the complete formation of Pd-*PTA* complexes. Hence, in the calculation only complex formation between Pd-*PTA* and ligand *A* is considered and each of these systems could be treated as a binary one. The conductometric titration of the ternary complex of glycine was performed using a solution mixture prepared as that for the potentiometric titrations, but without $NaNO_3$. All titrations were performed with standardized NaOH solution (≈ 0.1 *M*) at 25 °C in an atmosphere of purified N_2 using a titration vessel described previously [27].

The calculation were carried out using the computer program MINIQUAD-75 [28] running on a IBM-386 computer. The model selected was that which gave the best statistical fit and was chemically consistent with the titration data. Table 1 summarizes the obtained results. The concentration distribution of various complex species existing in solution as a function of *pH* was obtained using the SPECIES program [29].

References

[1] Kirshener S, Wei KK, Fransis D, Bergaman JG (1966) J Med Chem **9**: 369
[2] Rosenberg B (1971) Platinum Met Rev **15**: 42
[3] Rosenberg B (1982) In: Spiro TG (ed) Nucleic acids metal ion interaction, vol 1, P1. Wiley, New York
[4] Rosenberg B (1979) In: Siegel H (ed) Metal ion in biological systems, vol 2. Marcel Dekker, New York, p 127
[5] Cleare MJ, Hydes PC (1979) In: Siegel H (ed) Metal ions in biological systems, vol 2. Marcel Dekker, New York, p 1
[6] Rosenberg B, Van Comp L, Trosoko JE, Monsoy VH (1969) Nature (Lond) **222**: 385
[7] Stone PJ, Kelman AD, Sinex FM (1974) Nature **251**: 766
[8] Rozencweig M, Vonhoff DO, Selrik M, Muggia FM (1977) Ann Intern Met **86**: 803
[9] Selouri SK, Garoufis AG, Hodjiliadis N (1987) Inorg Chim Acta 135, L23
[10] Pneumatikakis G, Hodjiliadis N, Tleophanides T (1978) Inorg Chem **17**: 915
[11] Hodjilaidis N, Pneumatikakis G (1980) Inorg Chim Acta **46**: 255
[12] Pneumatikakis G (1984) Polyhedron **3**: 15
[13] Pneumatikakis G (1983) Inorg Chim Acta **80**: 89
[14] Taqui Khan B, Bhatt J, Najmuddin K, Shamsuddin S, Annapoorna KJ (1991) J Inorg Biochem **44**: 55
[15] Shoukry MM, Hohmann H, Van Eldik R (1992) Inorg Chim Acta **198–200**: 187
[16] Shoukry MM, Kenawy IM, El-Haj IH (1991) Transition Met Chem **16**: 637
[17] Shoukry MM, Shoukry EM (1993) Collect Czech Chem Commun **58**: 1103

[18] Shoukry MM, Khairy EM, Saeed A (1988) J Coord Chem **17**: 305
[19] Shoukry MM, Mahgoub AE, Hosny WM (1987) Transition Met Chem **12**: 77
[20] Perrin DD (1979) Stability constants of metal-ion complexes, part B. Organic ligands. Pergamon Press, Oxford
[21] Shoukry MM (1992) J Inorg Biochem **48**: 271
[22] Shoukry MM (1993) Bull Soc Chem Fr **130**: 177
[23] Lim MC (1977) J Chem Soc 15
[24] Prue JC, Schwarzenbach (1950) Helv Chim Acta 995
[25] Welcher FJ (1965) The analytical uses of ethylenediaminetetra acetic acid, 4th edn. Van Nastrand, Princeton
[26] Bates RG (1975) Determination of pH-theory and practice, 2nd edn. Wiley Interscience, New York
[27] Shoukry MM, Hosny WM, Khalil MM (1995) Transition Met Chem **20**: 252
[28] Gans P, Sabatini A, Vacca A (1976) Inorg Chim Acta **18**: 237
[29] Pettit L (University of Leeds) (personal communication)

Received December 4, 1995. Accepted December 12, 1995